REMARKS

In the current office action, the Examiner issued a restriction requirement Under 35 USC 121 and 372. The Examiner stated that the pending applications contained 2 groups of inventions that were not so linked as to form a general inventive concept under PCT Rule 13.1 bas on the Chen reference (US 2003/0096277). The Examiner issued a restriction requirement as to: Group I (claims 1-37) directed to methods for multiplex primer-based amplification; and Group II (claims 69 and 70) drawn to methods of diagnosing and differentially diagnosing the presence of a disease in a subject comprising subjecting a sample from the subject to multiplex primer-based amplification.

The applicant elects the claims of Group I (claims 1-37) for further prosecution on the merits. This election is made **with traverse.**

In addition, the Examiner issued a species restriction with regard to the claims of Group I. The Examiner identified 4 species: 1) (i) the concentration of target enrichment primers being used at the same concentration or (ii) at least one of the target enrichment primers being used at a higher concentration; 2) (i) the concentration of target amplification primers being used at the same concentration or (ii) at least one of the target amplification primers being used at a higher concentration; 3) the agent being detected being a (i) virus or (ii) bacteria; and 4) the detection method being a (i) direct or (ii) indirect detection method.

In response to the election of species requirements, the applicant elects the following species **without traverse**:

With regard to Species 1, the applicant elects (ii)- at least one of the target enrichment primers used at a higher concentration; claim 12 reads on this species.

With regard to Species 2, the applicant elects (ii)- at least one of the target amplification primers used at a higher concentration; claims 14 and 15 read on this species.

With regard to species 4, the applicant elects (i)- direct detection methods; claims 31-35 read on this species.

In response to the election of species requirements, the applicant elects the following species **with traverse**:

With regard to Species 3, the applicant elects (i)- virus; claim 24 in part and claim 25

read on this species.

Arguments Regarding Traverse

The applicant respectfully disagrees with the characterization of the general inventive concept and special technical feature characterizing the claims. The applicant submits that the disclosed method of amplification is unique and can be distinguished from other amplification methods known in the art.

With regard to Chen, the applicant would like to point out the difference between the primer configurations of Chen and the instant application. In Chen, the primary primers designated 10 in FIGS. 1 and 2 are equivalent to the second set of target enrichment primers (designated F_{in} and R_{in}). The primary primers of Chen comprise a specificity domain 11 and an artificial domain (designated 12). The specificity domain contains an allele element (designated 13) on the 3'end and a target element (designated 14) on the 5' end. The artificial element 12 contains a coupling element (designated 15) and a connecting element (designated 16); it should be noted that the coupling element 15 is distinct for each given allele (see paragraph 32, bottom of page 3 and top of page 4). Therefore, the artificial element 12 is distinct for each allele target. This distinctness leads to problems in designing multiplex PCR reactions (such as differences in Tm).

In contrast, the second set of target enrichment primers in the present specification contain first and second binding tags that do not contain an equivalent "variable sequence" corresponding to the coupling element 15 in Chen. The first and second binding tags are identical for each and every target sequence amplified. This feature provides an advantage in the amplification reactions described and is not taught or suggested by Chen.

Likewise, the portion of the second set of target enrichment primers that bind the nucleic acid containing the target sequence do not contain a 3' mismatch as do the primers of Chen.

Furthermore, Chen does not teach or suggest the use of outside primers (designated as the first set of amplification primers or F_{out} and R_{out} in the present application). The location of the primers as set forth in the current specification is provided in FIG. 1A; in FIG. 1A, a first set of target enrichment primers (designated F_{out} and R_{out}) and a second set of target enrichment primers (designated F_{in} and R_{in}) bracket

the target sequence. The second set of target primers are proximal to the target sequence and are themselves bracketed by the first set of target enrichment primers. Chen does not teach or suggest this configuration and applicant respectfully submits that such a configuration and its benefits were not previously anticipated in the art.

Chen simply states that different primer sets can be used to detect and differentiate between different allelic variants of a nucleic acid sequence; however, each of the primer sets binds to the same location on the nucleic acid and binds to the same target sequence (excepting the site of the allelic variation) (see FIGS 1 and 2 and paragraphs 0032-0052 of Chen). FIGS. 1 and 2 of Chen demonstrate clearly that Chen did not anticipate the use of primers equivalent to the "first set of target enrichment primers" as set forth in the current specification and claims.

Therefore, Chen does not teach or suggest then beneficial properties the claimed amplification methods of the present disclosure. As stated in the specification, the first set of target enrichment primers provide for superior amplification of the target sequence. Such an effect was not previously noted in the prior art. The combination of inside and outside primers in combination with the superprimers is new and novel. The data in attached Tables 1A and 1B and 2 indicates the increased amplification efficiency of the disclosed methods when the first set of target amplification primers are added.

The presence of the outside primers/first set of amplification primers leads to increased sensitivity in the present disclosure. Table 1 shows the results from a respiratory panel using the methods of the present disclosure. The reaction was performed essentially as described in Example 5 of the current application with the exception that in Table 1B the first set of target enrichment primers were omitted. As stated in the application, the rows indicate the identity of the nucleic acid used in the amplification step and the columns indicate the identity of the detection oligonucleotide used (the reaction mixture contained primers for all target sequences to be detected). As can be seen in a comparison of Table 1A (first set of target enrichment primers present) and Table 1B (first set of target enrichment primers absent), the omission of the first set of target enrichment primers significantly diminished the ability of the second set of enrichment primers and the target amplification primers to amplify the desired target sequence for detection. For example, in row 2 (RSVA), the inclusion of the first set of

target amplification primers resulted in a detection value of 1837; this value was reduced to 836 when the first set of target enrichment primers were omitted.

Table 1A- 1/100 dilution of isolated nucleic acid with outside primers (F_{out} and R_{out})								
Sample	SARS2	RSVA	PIV1	PIV3	MPN	CPN	ADV3-7-21	ADV4
SARS	**1600**	32	31	110	11	91	39	24
RSVA	39	**1837**	29	186	19	135	0	13
PIV1	15	18	**626**	119	17	93	83	13
PIV3	3	10	12	**329**	9	76	24	16
MPN	15	5	9	108	**229**	83	25	16
CPN	43	28	19	125	12	**143**	61	10
ADV3	39	14	19	138	14	92	**1973**	16
ADV4	11	14	14	108	1	69	15	**392**
ADV7	32	17	19	116	10	95	**958**	13
ADV21	18	7	18	89	10	56	**1967**	2

Table 1B- 1/100 dilution of isolated nucleic acid with no outside primers (F_{out} and R_{out})								
Sample	SARS2	RSVA	PIV1	PIV3	MPN	CPN	ADV3-7-21	ADV4
SARS	**969**	**11**	13	52	12	40	19	11
RSVA	0	**836**	**11**	63	6	42	18	13
PIV1	14	13	**216**	51	16	29	20	27
PIV3	18	14	**11**	**140**	10	41	12	11
MPN	11	13	6	61	**155**	43	21	11
CPN	19	10	12	58	9	**82**	23	3
ADV3	8	10	18	60	11	47	**1097**	12
ADV4	17	9	15	62	8	49	15	**201**
ADV7	14	11	12	54	5	41	**587**	6
ADV21	19	10	17	41	11	35	**1138**	12

The results are even more striking when the concentration of nucleic acid used as the template is decreased as shown in Table 2. The reaction was performed essentially as described in Example 5, with the exception that in one set of reactions (indicated by dark gray shading) the first set of target enrichment primers were omitted. As above, the rows indicate the identity of the nucleic acid used in the amplification step and the columns indicate the identity of the detection oligonucleotide used (the reaction mixture contained primers for all target sequences to be detected). In table 2, the B-samples indicate unknown clinical isolates obtained from a major southeastern research university. The legend below Table 2 indicates the occurrence of the genes to be detected in each sample as indicated by + or -. As can be seen in Table 2, the omission of the first set of target enrichment primers significantly diminished the ability of the second set of amplification primers and the target enrichment primers to amplify the desired target sequence for detection. The effect was greater when the concentration of

Table 2							
Sample	Conc	tuf	tuf	epi	epi	haem	haem
S. epidermidis	1.0 ng	3324	2033	4468	2850	18	13
	0.1 ng	2413	745	2945	1524	13	20
S. haemolyticus	1.0 ng	2616	270	25	26	3150	667
	0.1 ng	789	49	43	16	1457	82
S. hominis	1.0 ng	3182	481	17	23	12	34
	0.1 ng	1689	388	21	57	23	33
S. simulans	1.0 ng	2220	526	18	28	16	37
	0.1 ng	1462	50	18	27	35	41
B-155	n/a	2508	582	529	59	2530	685
B-184	n/a	3574	1525	3515	1560	17	38
B-113	n/a	1659	375	25	16	35	41
B-189	n/a	1840	37	89	31	10	25

Legend for Table 2

	tuf	epi	haem
S. epidermidis	+	+	-
S. haemolyticus	+	-	+
S. hominis	+	-	-
S. simulans	+	-	-

B series are clinical isolates from a Southeast research university

In addition, the claims of Group II rely on the amplification methods defined in claims 1 and 37 of Group I.

With regard to the species election, applicant asserts as above that all claims share the special technical feature comprising the arrangement and configuration of the primers disclosed, as supported by the data presented in Tables 1 and 2. As a result, Applicants respectfully submit the species election with regard to Species 1-4 should be removed. PCR Rule 13.3 states "Subject to Rule 13.1, it shall be permitted to include in the same international application a reasonable number of dependent claims, claiming specific forms of the invention claimed in an independent claim, even where the features of any dependent claim could be considered as constituting in themselves an invention."

In addition, with regard to Species 3, the applicant submits that the nature of the target to be detected does not impact the amplification methods disclosed. Nucleic acid isolated from a bacteria and virus are equally subject to the methods as described in the presented claims. Applicant respectfully submits the species election with regard to Species 3 be withdrawn.

CONCLUSION

For all the reasons given above, the Applicants respectfully submit that the amendment to the claims define and distinguish over the prior art currently of record. Applicants respectfully request the application be processed for allowance.

Respectfully Submitted,

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